

Replacement Sheet

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saturated fatty acid in most vegetable oil are 16:0 (palmitic acid) and 18:0 (stearic acid).

In the vegetable oil market, oil having less than 7% saturated FA content can be labeled "low-sat" and oil 5 having less than 3.5% saturated FA content can be labelled "no-sat". Canola (*Brassica napus*) seed oil is typically low in saturated fatty acid, but it is difficult to keep the saturated fatty acid level below the "low-sat" threshold of 7% saturated FA content.

10 Previous attempts have been made to address this problem. For example, transgenic plants have been made that contain heterologous plant genes involved in fatty acid metabolism (see for example: Shah S, Weselake R (2003) Farming For the Future, AARI project #19990032, Final 15 Report, pp.1-82; and Yao et al. Plant Biotech J 2003, 1:221). However, these transgenic plants showed little or no reduction of saturated fatty acid in the transgenic plant. For example, Yao et al. (2003) report a 1 to 2% decrease in saturated FA level associated with expression of 20 the ADS1 gene from *Arabidopsis* in *B.juncea* seeds.

In this context, prokaryotic genes provide an attractive alternative to plant genes, however prokaryotic proteins often show limited or no activity in a plant background (see e.g. Hahn JJ, Eschenlauer AC, Narrol MH, 25 Somers DA, Srienc F (1997) Growth kinetics, nutrient uptake, and expression of the *Alcaligenes eutrophus* poly(β -hydroxybutyrate) synthesis pathway in transgenic maize cell suspension cultures. Biotech Prog 13: 347-354).

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72); phaseolin promoter; and zein promoter. It may be possible to use a promoter that is not seed-specific, but such a promoter may not be as effective at reducing the saturated FA content of plant seed oil product.

5 In the present example, the coding region is also operably linked at the 3' end with the rbcs3' transcription terminator as a regulatory sequence. Other useful 3' regulatory regions which can also be used in the present invention include, but are not limited to: nopaline .0 synthetase polyadenylation region (NOS) and octopine polyadenylation region (OCS).

The DNA construct may be conveniently built in a first vector suitable for propagation in a bacterial host, then excised and ligated into a second vector for .5 introduction into a plant host. Examples of suitable vectors for introduction into a plant host include the pCAMBIA series of vectors (Center for the Application of Molecular Biology to International Agriculture (CAMBIA)) and the pBI series of vectors (BD Biosciences Clontech), as well .10 as pKYLX71-based vectors (Scharld et al. (1987)). Choice of vector will depend in part on the intended mechanism of transformation, i.e. Agrobacterium mediated transformation or direct gene transfer.

TRANSFORMED AND TRANSGENIC PLANTS AND PLANT CELLS

25 Transformed plant cells and transgenic plants comprising the nucleic acid of the invention can be generated using any methods of DNA delivery known to those skilled in the art (see for example "Plant genetic transformation and gene expression; a laboratory manual",

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EXAMPLE 6: CHARACTERIZATION OF TRANSFORMANTS:

Regenerated plants were identified as transgenic by polymerase chain reaction (PCR) using *des9* gene specific primers. Embryos of T1 seeds from regenerated transformed 5 plants were chopped into smaller pieces and placed in a selection plate containing kanamycin. Embryos from transgenic plants were either all green or a combination of green and pale (the ratio depending upon the number of transgenes integrated) while seeds from non-transgenic 10 plants were all pale. This was because the binary vector was engineered to carry a neomycin phosphotransferase (NptII) gene in tandem with the *des9* gene.

Integration of the *des9* gene into the canola genome was confirmed through Southern blot analysis. Genomic 15 DNA from young leaves was isolated following Dellaporta et al. (1983). Ten µg of genomic DNA was digested with a restriction enzyme that cut only in one end of the expression cassette in the binary vector. The digested DNA was then electrophoresed on a 1% agarose gel, transferred to 20 nylon membrane following the manufacturer's instruction (Amersham Canada Ltd., Oakville, ON) and probed with *des9* gene labeled with [α -32P]-dCTP by random prime labeling (Life Technologies, Grand Island, NY). Hybridization and washing of the blot at 65 °C was performed following Sambrook 25 et al. 1989.

Expression of the *des9* gene in the transgenic canola plants was confirmed through RNA analysis by RT-PCR and Northern blot. Total RNA was extracted from young leaves following procedure described in Verwoerd et al. (1989). The 30 RNA was electrophoresed on a formaldehyde-containing agarose